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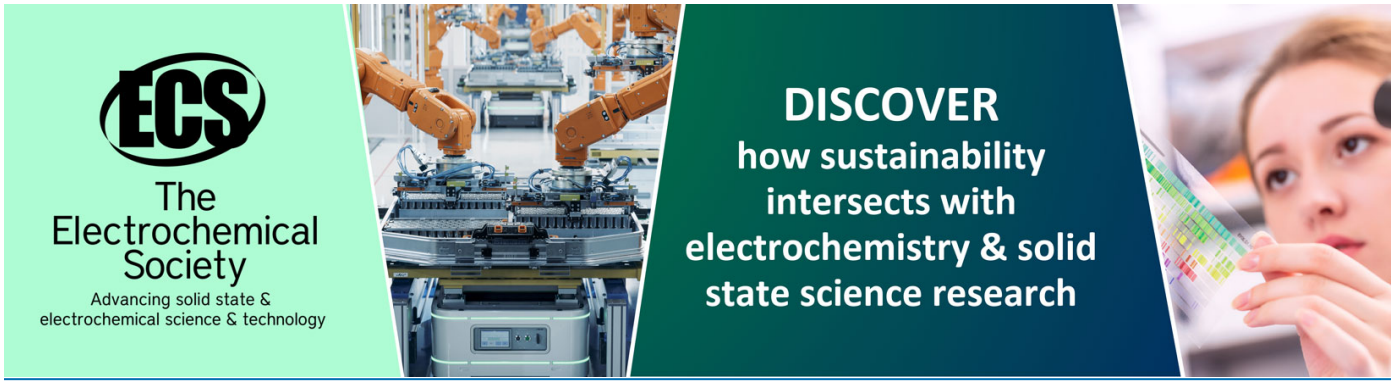

Manzamine C, an alkaloid indole as an inhibitor of the cancer cells adapted to nutrient starvation, from an Indonesian marine sponge of *Xestospongia muta*

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Abstract. Research of Manzamine C, Indole Alkaloids as cancer cell inhibitors in nutrient starvation, from the Indonesian Marine Sponge *Xestospongia muta* was done on May 2018. The goal of this study is to determine the cytotoxic activity of metabolite compounds of *Xetospongia muta* under glucose starvation. The active metabolite isolated using bioassay-guided separation with several steps of chromatography. Interpretation of IR data indicates that the active metabolites have the functional group of secondary NH_2 at 3435.56 cm^{-1}, CH methyl at 2853.39 cm^{-1}, CH methylene at 2769.64 cm^{-1} and CN imine at 1637.27 cm^{-1}. The LCMS-ESI analysis showed that the active metabolite has a molecular weight $[M+H]^+$ 347.445 m/z which indicates the active metabolite has a molecular formula $C_{23}H_{29}N_3$. The activity of metabolite compounds against PANC-1 cells showed cytotoxic activity under glucose starvation conditions with values IC_{50}=10 μM, whereas no growth inhibition was observed up to 100 μM under the general culture conditions.

1. Introduction

Cancer is a disease caused by the destruction of the basic regulatory mechanisms of cell behavior, especially the mechanism of cell growth and differentiation. Cancer occurs because of an error or failure in the condition of cells that result in uncontrolled growth factors. The process of cancer occurrence is called carcinogenesis which begins to increase the proliferation of cells undergoing genetic mutations resulting in excessive cell reproduction [1,2,3,4].

Cancer therapy can be done in various ways, ranging from the conventional nature of the surgery to the modern nature of the use of chemotherapy, radiation, hormones, and monoclonal antibodies. However, chemotherapy has some disadvantages and harmful side effects, including causing damage to surrounding tissues and other organs, such as the stomach, liver, and kidney, in addition to the expensive and long treatment time. The rapid development of in vitro methods makes it easier to screen for crops or anticancer active compounds prior to testing in vivo using experimental animals [5,6,7,8].

From the recent biological studies of the cancer cells adapt- ed to the nutrient starvation, the activation of phosphoinositide 3-kinase (PI3k)/mammalian target of rapamycin (mTOR)

signaling pathway and the unfolded protein response (UPR) such as induction of glucose-related protein 78 (GRP78) were found to be important for the adaptation of cancer cells to nutrient starvation, and these processes have attracted attention as drug targets for cancer chemotherapy [9,10,11,12,13].

Following this background, we established a screening system to search selective growth inhibitors against the cancer cells adapted to the nutrient-starved conditions by utilizing the glucose deficient culture medium. As a result of screening from the marine medicinal resources and bioassay-guided separation, a manzamine alkaloid named manzamine C (1) was isolated from an Indonesian marine sponge of *Xestospongia muta*. In this paper, the isolation, structure elucidation and biological activity of manzamine C (1) are presented.

2. Materials and Methods

2.1 Extraction and isolation

Sponges 100 g (dry weight) were cut into small pieces and extracted using methanol for 72 hours. The results obtained are then evaporated to produce 32.8 grams of crude. The results are then partitioned using a mixture of *n*-Hexane: ethyl acetate : ethanol (1: 1: 1 v / v) resulting in an ethanol fraction (21.73 g).

Based on bioactivity guidance, methanol fraction [21,73 g, IC_{50} (Glucose Deficiency Media) = 0.8 μg/mL, IC_{50} (General Glucose Media = 3.0 μg/mL). Futhermore, the extract of ethanol fraction (21.73 g) was fractionated by open column chromatography (OPN-C18) with ethyl acetate: ethanol: trifluoroacetate 0.1% gradient resulting in four different fractions on the KLT plate. The activity of the third fraction [7.88 g, IC_{50} (Glucose Deficiency Media) = 0.1μg/mL, IC_{50} (General Glucose Media = 3.0 μg/mL) is further purified by HPLC in column RP-18 with eluent MeCN: H_2O: trifluoroacetate 0.1% gradient resulting in seven fractions. The activity of the fourth fraction [2.74 g, IC_{50} (Glucose Deficiency Media) = 0.2 μg/mL, IC_{50} (General Glucose Media) = 3.0 μg/mL] showed cytotoxic activity against PANC-1 cells in glucose deficiency medium.

2.2 Materials

Biorad spectroscopy, biorad microplate rider, plastic plate 96-well, incubator CO_2, rotary evaporator dan centrifuge. Kanamycin 50 ug/mL, trypsin, Mc-Coys medium, trypan blue, panc-1 human cell, general glucose medium, glucose deficiency medium, , phosphate buffered saline (PBS), methanol, ethanol, ethyl acetate, supplement Dulbecco's Modified Eagle's Medium (DMEM), Foetal Bovine Serum (FBS), *n*-Heksane and aquadest.

2.3 Pancreas cell culture

Human pancreatic cell carcinoma (PANC-1) is grown on Dulbecco's modified Eagle's medium (DMEM) supplemental medium, which is supplemented with 10% bovine serum fetus (FBS) and kanamycin (50 μg/mL) at 37°C under 5% CO_2 atmospheric conditions [14,15].

Pancreatic cells in starvation nutrition condition, PANC-1 cells are cultured in Glucose Deficiency Medium [Basal Medium (25 mM N- (2-hydroxyethyl) piperazine-N'-2-ethanatulfonic acid (HEPES) buffer (pH 7,4)]. Supplemented with 6.4 g/L NaCl, 700 mg/L $NaHCO_3$, 400 mg/L KCl, 265 mg/L $CaCl_2 \cdot 2H_2O$, 200 mg/L $MgSO_4 \cdot 7H_2O$, 125 mg/L NaH_2PO_4, 0.1 mg/L $Fe(NO_3) \cdot 9H_2O$, 15 mg/L Phenol red, 10 mL/L MEM solution of vitamin (X100), 200 mmol/L L-glutamine solution (GIBCO) 50 mg/L kanamycin) containing 10% FBS dialectate. General glucose medium [Basal medium equipped with 10% FBS and 2.0 g/L

glucose (final concentration of 25 mM)] is also used for bioassay as a general culture condition for comparing sample activity under starvation nutrition condition [16,17,18].

3. Results and Discussions

3.1 Cytotoxic test

The PANC-1 cell (1×104 cell/100 µL in plastic plate 96-well) was incubated in a DMEM supplement medium with 10% FBS for 24 h. DMEM supplements are then replaced with Glucose Deficiency Medium and General Glucose medium. After 12 hours of incubation, the sample was added and incubated for 12 hours at 37°C under atmospheric pressure containing 5% CO_2.

The results of cytotoxic tests showed that the viability rate of PANC-1 cells had differences in general glucose medium and glucose deficiency medium. Observation of cytotoxic effects using cell proliferation detected by WST-8 colorimetric reagents. The value of IC_{50} was determined by the linear interpolation of the viability growth curve. Based on IC_{50} values indicate differences in viability in general glucose medium and glucose deficiency medium. The active compound has anti-proliferative activity on glucose deficiency medium but does not show activity in general glucose medium.

Cell proliferation was detected by WST-8 colorimetric reagents. The value of IC_{50} is determined by the linear interpolation of the growth-survival curve. Based on IC50 values indicate differences in viability in glucose deficiency medium and general glucose medium. The active-compound had anti-proliferative activity in glucose deficiency medium but did not show activity in general glucose medium [19, 20, 21].

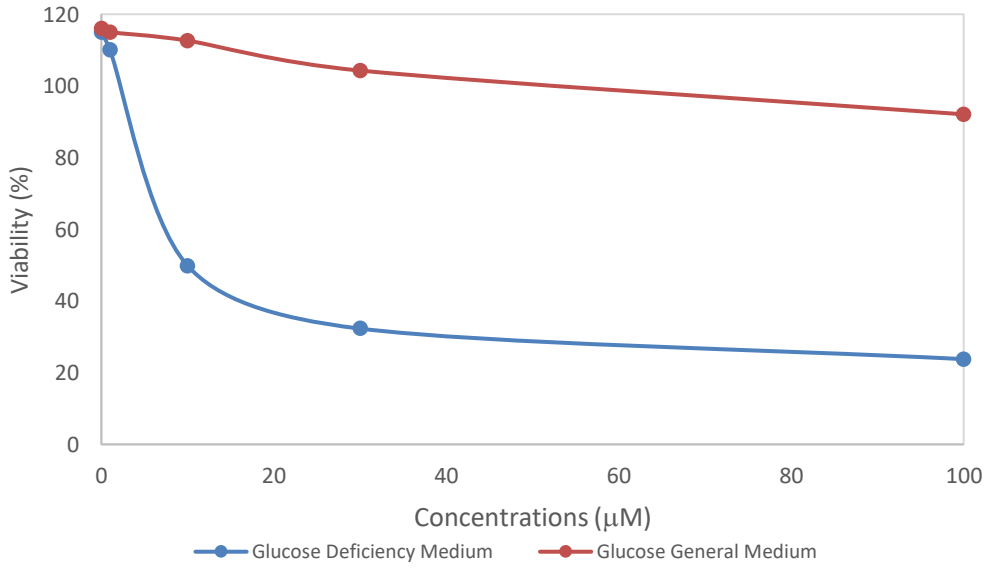

Figure 1. Effect of glucose concentration on cytotoxic activity against PANC-1 cells

Based on the interpretation of IR data indicates that it is found that bioactive metabolites belong to an alkaloid compound group. The active metabolite can inhibit the overall

performance of Cyclin-Dependent Kinase (Cdk) which is a cell cycle regulator. The mechanism of interaction of alkaloid compounds lies in the inhibition of the action of Cdk-activating kinase (CAK) enzyme that inhibits the formation of active Cdk-cyclin complexes. Alkaloids can bind to protein kinases on their ATP-binding sites [13,14,15].

The receptors in G1/S and in G2/M were disturbed by the presence of alkaloid compounds that inhibit the signal transduction process from growth factors. Alkaloids are able to inactivate proteins that play a role in signal transduction, such as tyrosine kinase so as to have the effect of blocking growth factor receptors, inhibiting mitogen-activated protein kinase (MAPK), on the tyrosine kinase receptor signal pathways (RTKs) [16,17,18,19].

We assessed the selectivity of anti-proliferative activity (S.I.) on the basis of IC_{50} value differences in general glucose medium and glucose deficiency medium.

3.2 Structure Identification

Compound 1 (colorless solid) which has been isolated known to has the molecular formula $C_{23}H_{29}N_3$ determined by LCMS-ESI with molecular weight $[M+H]^+$ 347,445 m/z. The active-compound has a double bond equivalent (DBE) value is 6. Interpretation of FTIR spectrum indicates functional groups of secondary NH_2 at 3435.56 cm^{-1}, CH methyl at 2853.39 cm^{-1}, CH methylene at 2769.64 cm^{-1} and CN imine at 1637.27 cm^{-1}. The ^1H and ^{13}C NMR spectrum indicate that the active metabolite has 29 hydrogen atoms and 23 carbon atoms.

Table 1. NMR spectrum of alkaloid indole compound

Position	δ_H	δ_C	Mult. (*J*), int
1	7,91	112.7 (d)	m, 1H
2	8,64	137.3 (d)	m, 1H
3	-	147.7 (s)	-
4	-	136.8 (s)	-
5	-	143.8 (s)	-
6	7,63	111.1 (d)	m, 1H
7	7,5	121.7 (d)	m, 1H
8	7,2	119.8 (d)	m, 1H
9	8,19	121.4 (d)	m, 1H
10	-	123.3 (s)	-
11	-	127.3 (s)	-
12	2,98	27.8 (t)	m, 2H
13	2,69	31.9 (t)	m, 2H
14	2,34	59.8 (t)	m, 2H
15	1,37	23.1 (t)	m, 2H
16	1,31	25.4 (t)	m, 2H
17	1,94	25.7 (t)	m, 2H
18	5,42	130.1 (d)	m, 1H
19	5,42	130.1 (d)	m, 1H
20	1,94	25.7 (t)	m, 2H
21	1,31	25.4 (t)	m, 2H
22	1,37	23.1 (t)	m, 2H

23	2,34	59.8 (t)	m, 2H

^{1}H dan ^{13}C (500 MHz) data NMR ($(CD_3)_2SO$)

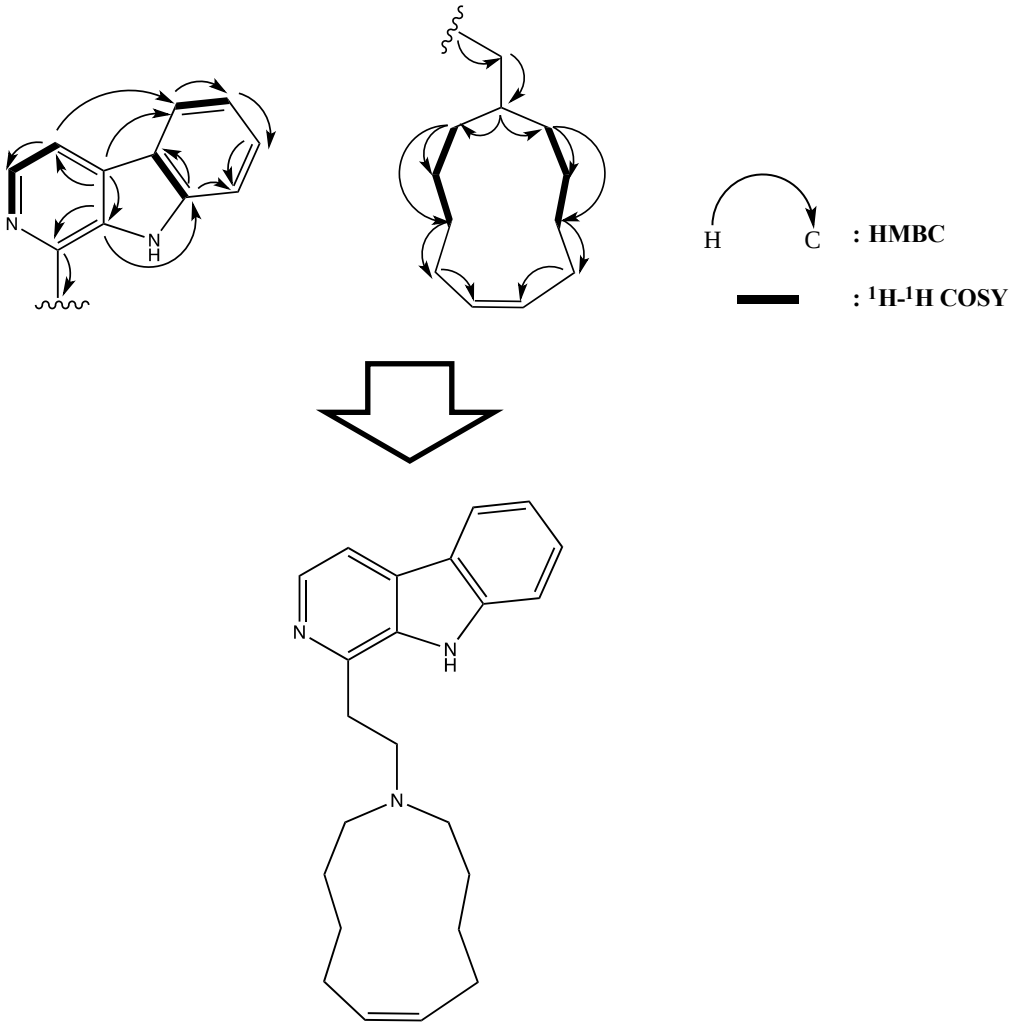

Figure 2. Manzamine C

The ^{1}H spectrum (Table 1) showed six CH methylene signals (δ_H = 1.31, 1.37, 1.94, 2.34, 2.69, 2.98 ppm). The proton signals from the amine groups (δ_H = 11.70 ppm) indicates as a secondary amine. Based on the data indicates that the compound component as the indole alkaloid skeleton.

The ^{13}C spectrum (Table 1) showed four equal secondary carbon signals at C15-C22, C16-C21, C17-C20, C14-C23 (δ_C = 23.1, 25.4, 25.7, 59.8 ppm) so it shows in the same neighbourhood. Whereas the two secondary carbon signals are not the same as C12 and C13 (δ_C = 27.8 dan 31.9 ppm) so it shows in the different neighbourhood.

Two tertiary carbon signals that show the same neighbourhood at C18-C19 (δ_C = 130.1 ppm) and six tertiary carbon signals residing in different neighbourhoods are present at C6, C1, C8, C9, C7 and C2 (δ_C = 111.1, 112.7, 119.8, 121.4, 121.7, 137.3 ppm). And five quartz

carbon signals at C10, C11, C4, C5 and C3 (δ_C = 123.3, 127.3, 136.8, 143.8, 147.7 ppm). Based on the interpretation of data indicate that the active metabolite as *manzamine C.*

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